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PREVALENCE AND RISK FACTORS OF MDR-TB IN WEST ARMACHIHO AND METEMA WOREDAS, NORTHWEST ETHIOPIA

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LIST OF ABBREVATIONS

AFB	Acid-Fast Bacillus
AFS	Acid Fast Stain
AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
CXR	Chest x-ray
DOTS	Directly Observed Treatment, Short-course
DR-TB	Drug-resistant tuberculosis
DRS	Drug resistance surveillance or survey
DST	Drug susceptibility testing
EH	Ethambutol and Isoniazid
EHNRI	Ethiopian Health and Nutrition Research Institute
EPTB	Extra pulmonary TB
FM	Florescent Microscopy
TB-HBC	Tuberculosis High-burden countries
HC	Health center
HIV	Human Immunodeficiency Virus
INH	Isoniazid
RIF	Rifampicin
RHZE	Rifampicin Isoniazid Pyrazinamide Ethambutol
LPA	Line-probe assay
LJ	Lowenstein Jensen
MDR	Multi-drug resistant TB
MOH	Ministry of Health
PC	Probe check
PCR	Polymerase Chain Reaction
PLWHA	People living with HIV/AIDS
PTB	Pulmonary TB
SPC	Specimen processing control
SMS	Spot-Morning-Spot

WHO	World Health Organization
XDR-TB	Extremely drug-resistant tuberculosis
X-PERT	Gene X pert
ZN	Ziehl-Neelsen stain

ABSTRACT

BACKGROUND: Multi drug resistant tuberculosis (MDR TB) is an emerging challenge for TB control programs globally. According to the National DRS result in 2005, 1.6% of new cases and 11.8 % of previously treated cases wereMDRTB. According to WHO report on the prevalence of MDR-TB in 2012,Ethiopia stands 15th out of the 27 high priority countries in the world and 3rd in Africa following South Africa and Nigeria.

OBJECTIVES: The aim of this study was to assess the prevalence of MDR-TB and associated risk factors in West Armachiho and Metema woredas of North Gondar.

METHODS: A cross-sectional study was conducted in West Armachiho and Metema woredasbetweenFebruaryandJune 2014. All smear positive pulmonary tuberculosis patients wereincluded in the study.Socio-demographic and risk factor data were collected using a semi-structured questionnaire. Informed consent was obtained from study subjects and two morning sputum samples were collected prior to starting anti-TB treatment for culture and drug susceptibility testing. Culture was performed on Lowenstein Jenson Medium (LJ). Drug susceptibility testing wasfirst performed for rifampicin using GeneXpertMTB/RIF. For those rifampicin resistant strains, DST was performed for both isoniazid and rifampicin to identify MDR-TB using proportional method on LJ media. Finally, data wasentered, cleared and analyzed using statistical Package SPSS version 20. Tables and graphs were used to describe the findings. Logistic regression was used to assess the association. P-value and 95% confidence interval werealso used to assess the statistical significance.

RESULTS: Of 124 smear-positive pulmonary TB patients, 117 (94.4%) were susceptible to Rifampicin, while 7(5.7%) were confirmed to be resistant to Rifampicin and Isoniazid. The overall prevalence of MDR-TB was 5.7%(2.3% among new cases and 13.9% among previously treated cases).History of previous treatment(OR=7, P=0.025) wassignificantly associated risk factor for MDR-TB.

CONCLUSION AND RECOMENDATION: The overall prevalence of MDR-TB among new and previously treated cases (5.7%) was considerably high. History of previous treatment was risk factor for MDR-TB. Therefore, efforts to reduce the burden of MDR-TB such as early case detection and treatment of MDR-TB, strengthening TB infection control activities and proper implementation of DOTS should be made in the study area.

Key Words: Tuberculosis, MDR-TB, Risk Factors

1. INTRODUCTION

1.1Background

Tuberculosis is a major public health problem throughout the world. About a third of the world'spopulation is estimated to be infected with tubercle bacilli and hence at risk of developing active disease. According to the WHO Global TB Report 2012, there were an estimated 8.7 million incident cases and 12 million prevalent cases of TB globally, about 26% of the incident TB casesoccurred in Africa in 2011 (1).

Twenty twoHigh Burden Countries (HBCs) have been given highest priority at the global level since 2000 which accounted for 82% of all estimated cases worldwide. Ethiopia is one of the 22 HBCs. According to the national population based TB prevalence survey conducted in 2010/11 the prevalence of smear positive TB among adults and all age groups was found to be 108 and 63 per 100,000 populations, respectively (2).WHO global TB report in 2012 estimated the prevalence of all forms of TB was 200,000 (237 per 100,000 populations). Among these 15,000 deaths (18 per 100,000 populations) was due to tuberculosis (1-2).

Multidrug-resistant (MDR)tuberculosis (TB) caused by *Mycobacterium tuberculosis* strains resistant to at least isoniazid and rifampin has emerged as a global epidemic resulting largely from deficiencies in TB case management and program management (3). Approximately 425,000 MDR-TB cases occur annually worldwide, representing nearly 5% of the world's annual TB burden (4). Patients with MDR-TB require a much longer treatment period, usually 24 months, compared with the 6–8 months required for drug-susceptible TB (5). Additionally, treatment requires the use of "second- line" drugs, which are more toxic and prohibitively more expensive, with MDR-TB drug costs alone that are, on average, 100 to 300-fold higher than those associated with drug-susceptible TB.Furthermore, patients with MDR-TB have lower cure rates and higher mortality than do patients with drug-susceptible TB (5). In well-performing MDR-TB programs in settings with a low HIV infection prevalence, treatment success is generally 70%–80%; by comparison, treatment success for drug-susceptible TB in well-performing TB programs can exceed 90% (6).

Resistance to the second line drugs also arise then the disease becomes virtually untreatable. Extensively drug resistant-tuberculosis (XDR-TB) has been reported in all regions of the

world (7). XDR-TB is defined as resistance to at least rifampicin, isoniazid, any of the second line injectable drugs (capreomycin, kanamycin or amikacin) and any of the fluoroquinolones (8). Control of drug resistant tuberculosis requires a strong health infrastructure to ensure the delivery of effective therapy coupled with surveillance and monitoring activities to enable timely intervention to limit transmission and spread of the disease(9).

Drug-resistant TB can be primary or secondary(acquired). Primary resistance is caused by person-to-person transmission of drug-resistant organisms. Secondary resistance is drug resistance in a patient who has received at least one month of anti TB treatment. Prior exposure to anti-TB drugs is a well-established risk factor for drug resistance. In previously treated patients, the probability of any resistance was over 4-fold higher, and of MDR-TB over 10-fold higher, than for untreated patients. Among countries with a high burden of TB, previously treated cases ranged from 4.4% to 26.9% of all patients registered in directly observed treatment program. In the two largest high-TB burden countries (China and India) re-treatment cases accounted for up to 20% of sputum smear-positive cases (10).

Drug-resistant TB is a man-made problem, largely being the consequence of human error as a result of individual or combination of factors related to management of drug supply, patient management, prescription of chemotherapy, patient adherence, poor infection control practice, irrational drug use and generally poor Directly Observed Treatment (DOTS) implementation practice has been identified as a major contributing factor for the spread of DR-TB (Drug Resistance Tuberculosis). But spontaneous mutation of bacteria has also an effect for the progression to DR-TB. Drug resistance tuberculosis, like drug susceptible TB, is transmitted through inhalation of infected droplet nuclei (11).

1.2 Statement of the problem

1.2.1Global Burden of MDR-TB

Tuberculosis (TB) remains a major global health problem. In 2012, WHO estimates 8.6 million people developed TB and 1.3 million died from the disease (including 940,000 among HIV-positive and 320 000 deaths among HIV-negative people). Regarding MDR, in its estimation there were 3·7% MDR (range 2·1–5·2%) of new cases and 20% MDR (range 13–26%) of previously treated cases. The incidence of MDR-TB estimated to be 450,000 and the overall

proportion of deaths from MDR-TB is 5.7% of the number of TB deaths which is unacceptably large given that most are preventable. In eastern Europe and central Asia, 9–32% of new patients and more than 50% of previously treated patients have MDR tuberculosis (12).

The estimated proportion of MDR-TB for all countries was then applied to estimated new (incident) TB cases.(13), it is estimated that 489 139 cases emerged in 2006, and that the global proportion of resistance among all cases is 4.8% China, India and the Russian Federation are estimated to carry the highest number of MDR-TB cases. China and India carry approximately 50% of the global burden, and the Russian Federation 7 % (14).

The high rates of MDR-TB were observed in the 2 most populous Chinese and Indian provinces. In Henan Province, the most populous province in China, 11% of new cases had MDR-TB. In Tamil Nadu, India, 3.4% of new cases had MDR-TB, the prevalence of isoniazid resistance was 15%, and rifampicin resistance was found in 4.4 % (15).

1.2.1. Burden of MDR-TB in Africa

According to WHO estimation nearly 60,000 MDR-TB cases occur annually in the Sub-Saharan Africa region (14% of the global burden) (16). South Africa, with a nearly 20% HIV infection prevalence among persons 15–49 years of age, has 11,000 MDR-TB cases annually (2.4% of the global MDR-TB burden). Of these 1.8% was new TB cases. MDR-TB is more challenging and much more expensive to treat than drug sensitive tuberculosis. Eight other countries have an estimated annual MDR-TB incidence of 11,000 cases, including Cote d'Ivoire, Democratic Republic of Congo, Kenya, Malawi, Mozambique, Tanzania, Zambia, and Zimbabwe (17).

According to the WHO/IUATLD survey, countries such as Mozambique, Cote D'Ivoire, Cameroon, Argentina, the Dominican Republic, and Mexico are a concern because the prevalence of MDR-TB in new cases is 13%. The full magnitude of the problem is still unknown in a number of countries with high TB incidence, such as Democratic Republic of the Congo, Ethiopia, Nigeria, Indonesia, Bangladesh, and Pakistan (18).

The prevalence of MDR-TB among new patients increased from 0.2% to 0.8%, in Botswana, relatively high treatment default rates, uncontrolled use of second- line drugs for MDR-TB and

high prevalence of HIV infection among patients with TB overall (60%–83%), increases in MDR-TB(19).

In Kenya, with the fourth highest TB burden in the region, data from a 2002 national anti-TB drug–resistance survey indicating that 0.8% of new patients with TB without prior treatment and 3.6% of previously treated patients with TB had MDR-TB. This equates to a conservative estimate of nearly 2400 new patients with MDR-TB annually (20).

1.2.3. Burden of MDR-TB in Ethiopia

According to WHO 2012 report, Ethiopia stands 15th out of the 27 high priority countries in the world and 3rd in Africa following South Africa and Nigeria with (1600 and 480) among new and retreatment cases respectively wereMDR-TB cases (1).According to the drug resistance survey conducted nationwide in 2005 (EHNRI/FMOH), the prevalence of MDR-TB was: 1.6% in newly diagnosed TB and 11.8% among previously treated TB cases(21).The country's burden of MDR-TB in 2009 was estimated to be 1500(870-2600) and 420(230-740) among new and re-treatment cases respectively. Despite being a huge global threat, access to treatment is very limited with only 10% of the estimated MDR-TB cases among notified TB cases in 2009 in the high MDR-TB countries and 11% globally were enrolled on treatment (21).

A study conducted in North West of Ethiopia to assess the level and risk factors for first- and second-line drug resistance among tuberculosis (TB) patients shows an overall prevalence of 5.0% multidrug-resistant (MDR) from which 3.7 % were from new cases and 10.9% were from previously treated cases (22).

In West Armachiho and Metema woredas, information regarding TB treatment interrupters, relapses, and failure cases were not well documented. According to the national comprehensive TB/HIV and TBL guideline these are criteria for suspicion of MDR-TB. Moreover, as to our knowledge there are only limited data regarding MDR-TB in this particular study area. Therefore, the present study aimed to fill these gaps.

2. LITERATURE REVIEW

2.1 Prevalence of MDR-TB

A Population based cross-sectional study conducted in Georgia shows isolates recovered from 195 (14.8%) of 1314 patients had MDR-TB; with the prevalence of 6.8% in newly diagnosed cases and 27.4% in re-treatment cases. Previous anti-TB treatment and gender (being female) were risk factors for MDR-TB(23).

A cross-sectional, descriptive study In India shows: from a total of 196 pulmonary TB patients which were screened; MDR-TB was detected in 40 (20.4%) patients. The mean age of MDR-TB patients was 33.25. Of these 40 patients, 29 (72.5%) had relapse, 3 (7.5 %) had treatment failure and 8 patients (20%) were defaulters. Nine patients (22.5%) were female. Thirty six patients showed resistance to rifampicin and isoniazid; 4 patients showed resistance to streptomycin (in addition to rifampicin, isoniazid) (24).

Another study in India Showed thatof 218 cases, whose mean age of the patients was 27.8 and 59 (27%) were female, a prevalence of MDR-TB among new sputum positive pulmonary TB patients was 1.1%.two cases of MDR-TB were detected. Both were male, HIV negative, aged 20 and 25 yrs. and the resistance rates (%) observed to each first-line drugs of isoniazid and Rifampicin were 6.2, rifampicin 1.1, respectively (25).

A study from Korea showed that among 637 patients which were enrolled in the study; Resistance to at least one first-line drug was identified in 11.7% of new cases and 41.6% of previously treated cases. MDR-TB was detected in 3.9% of new cases and 27.2% of previously treated cases. Factors associated with MDR-TB were found to be age less than 45 yrs. and previous TB treatment. (26).

A Surveillance report in Gujarat state of India showed; Of 1571 patients enrolled, prevalence of MDR-TB was found to be 2.4%, 37 isolates from new patients, 173 (11%) had any INH resistance and. But from 1047 isolates from previously treated patients, 387 (37%) had any INH resistance and MDR-TB was found in 182 (27).

A five year retrospective study in India to assess drug resistance profiles of *Mycobacterium tuberculosis* isolates to first line anti-tuberculosis drugs shows a prevalence of MDR was 320

(47.54%) out of 673 *M. tuberculosis* isolates tested for drug sensitivity against first line drugs (28).

A study from Pakistan Karachi shows that, the prevalence of MDR-TB was 5.0 %,(new 2.3%, previously treated 17.9%); and female gender and prior history of incomplete treatment were found to be associated risk factors for MDR-TB(29).

A study conducted in Pakistan showed that 76/672 (11.3%) culture positive strains were resistant to one or more drugs. Primary MDR-TB was 1.8% (n=12) (30).

In a study carried out in South Africa showedof 119,218 notified TB cases, 2799 (2.3%) were multi-drug resistant (MDR).The two worst affected districts in the state were uMzinyathi where 226 (4.1%) of 5522 notified TB cases were MDR, and of these 120 (53%) were extensively drug resistant (XDR), and uMkhanyakude where 337 (4.8%) of 6991 notified TB cases were MDR, but, of these only 4 or (1.2%) were XDR. The worst affected medical centre was COSH where 164 or 9.8% of notified TB cases were MDR and of these 99 (60%) were XDR (31).

A study conducted in Nigeria showed that, the overall prevalence of multi-drug resistant *Mycobacterium tuberculosis* among TB patients was found to be 5.8%. MDR-TB was found to be significantly associated with HIV seropositive patients (32%) compared to HIV-seronegative (2.2%) patients (32).

A survey on primary anti-tuberculosis drug resistance conducted on smear positive pulmonary TB patients in Addis Ababa showed that, of173 patients enrolled, 21.4% were resistant to at least one drug. Single drug resistance was observed to isoniazid in 13.3%, to rifampicin in 1.2%. The prevalence of resistance to at least one drug was 15.7% and 23.7% among patients with and without HIV co-infection. Only one patient (0.6%) had a multidrug resistant (MDR) strain. However, the prevalence of resistance to more than one drug was 10.4% (33).

A retrospective study on the prevalence of MDR-TB among retreatment MTB cases conducted in St.Peter Hospital, Addis Ababa showed that, of the total 84 patients resistance to at least one drug was observed in 53.6% and 26.2% of the isolates were multi drug resistant (MDR)(34).

Another study in National TB Reference Laboratory in Addis Ababa showed that,of 107 *M. tuberculosis* isolates recovered and tested for drug susceptibility against first line anti-TB

drugs,MDR-TB was observed in one of the 44 new cases (2.3%) and 45/63 previously treated patients (71.4%)(35).

2.2 Risk Factors of MDR-TB

A research conducted in Honk Kong, China showed that from 156MDR-TB patients,the risk factors for acquiring the diseases were non-permanent residents, frequent travel and younger age (36).

Risk factors for resistance can be those facilitating the selection of resistance in the community and the specific conditions that appear to increase some patients' vulnerability to resistance. The epidemiological situations varies greatly across countries, principally due to poor treatment practices and poor implementations of control programme in the past and even today, to a lesser degree and recent data have suggested that national TB programme that use existing drugs efficiently can postpone and even reverse the MDR-TB epidemic. Factors' leading to the MDR-TB epidemic includes treatment failures with first-line rifampicin containing regimens, contacts of MDR-TB cases and patients previously treated for TB causesthe highest rates of resistance (37).

In A study conducted to detect risk factors for multidrug resistance in patients with pulmonary tuberculosis in four European Union countries: France, Germany, Italy, and Spain done between 1997 and 2000. A total of 138 cases and 276 controls were studied. Considering the four countries as a whole, the statistically significant risk factors were intravenous drug use; asylum-seeker; living in a nursing home; previous tuberculosis with pulmonary location; prison; known tuberculosis contacts; immunosuppression other than HIV/AIDS; current tuberculosis with pulmonary location; and health-care worker (38).

A study conducted in Spain to identify factors associated with multidrug resistance. Patients with positive culture for *M. tuberculosis* and with available drug-susceptibility tests were included. Age, gender, country of origin, homelessness, alcohol consumption, intravenous drug use, contact with a tuberculosis patient, sputum smear, previous tuberculosis treatment, HIV infection, history of imprisonment, diabetes mellitus and chronic obstructive pulmonary disease were assessed. Thirty patients with MDR-TB and 666 patients with non-MDR-TB were

included from the years 1997 to 2006. The factors associated with MDR- TB in multivariate analysis were previous tuberculosis treatment, age group 45–64 years and alcohol abuse (39).

Indian study in a tertiary Hospital to assess Risk Factors for MDR and XDR-TB such as HIV infection (tested in 134/203 (66%) cases), socioeconomic status, gender, age category, cigarette use, alcohol use, site of TB disease, diabetes, residence outside of the state (referral patients), cavitations on CXR, number of different previous treatment regimens, previous use of injectable agents and fluoroquinolones and adequacy of initial treatment (defined as whether or not the initial treatment regimen followed the regimen outlined by the national treatment program for new smear positive TB patients were assessed. Previous treatment with an injectable and fluoroquinolones was strongly associated with MDR (40).

A Population-based study on new and previously treated patients with TB collected within an international drug resistance surveillance network. Of 9615 patients, which were enrolled 8222 (85.5%) were new cases of TB and 1393 (14.5%) were previously treated cases. Compared with new cases, previously treated cases were significantly more likely to have resistance to two, primary anti TB drugs. A linear increase in the likelihood of having MDR-TBwas observed as the total time (measured in months) of prior anti-tuberculosis treatment increased ($P < 0.001$). In multivariate analysis, prior TB treatment for 6–11 months ($P < 0.001$) and ≥12 months ($P < 0.001$), but HIV positivity, was not associated with MDR-TB (41).

A study conducted in Namibia showed, of the enrolled 117 MDR-TB cases and 251 TB controls, among cases, 97% (113/117) had been treated for TB before the current episode compared with 46% (115/251) of controls. Cases were significantly more likely to have been previously hospitalized and to have had a household member with MDR-TB. These associations remained significant when separately controlled for being currently hospitalized or HIV-infection (42).

A retrospective study conducted in Nigeria indicates that out of the 88 patients who had drug-susceptibility test result; there were 50 males and 38 females. The multi-drug resistant TB (MDR-TB) rate was 76.4%. The only significant factor for the development of drug resistance and MDR was the history of previous anti TB treatment ($P<0.01$). Other factors such as age and gender were not significantly associated with drug resistance TB (43).

A cross-sectional survey conducted in Swaziland shows MDR strain was isolated in 27 new cases, resulting in MDRTB prevalence of 7.7%. Past TB treatment, being female, HIV infection, and age 28–40 years were significantly associated with MDR TB. Past TB treatment and HIV-infected patients were four times and two times more likely to be infected with an MDR TB, respectively. The youngest age group was close to being significantly associated with MDR TB regardless of HIV status and history of previous treatment (44).

A study focused on determinants of MDR-TB in Bangladesh shows younger age and, peri-urban localities were associated with MDR-TB. History of contact and tuberculosis in the past were four and eight times, a risk for MDR-TB, respectively, Regularity and always observation of treatment, sputum conversion negatively associated with multi-drug-resistant tuberculosis. Gender and socio-economic status did not show any influence (45).

A cross sectional study conducted in Jimma University specialized hospital, southwest Ethiopiaamong new cases of smear positive TB patients to determine the pattern of resistance to first-line drugs and risk factors. Multidrug-resistance TB (MDR-TB) was observed intwo patients (1.5%). No statistically significant difference in the proportion of resistance by sex, age, HIV status and history of beingimprisoned was observed (46).

A case control study conducted at St. Peter Hospital and five health centers in Addis Ababa showed that, of the total 134 cases and an equal numberof controls which were enrolled in the study, being male and history of previous treatment were risk factors for MDR-TB development. But HIVinfection was not significantly associated with the occurrence of MDR-TB (47).

3. SIGNIFICANCE OF THE STUDY

Tuberculosis case that has not been treated previously can harbor and expel bacilli resistant to one or more anti-tuberculosis drugs (primary resistance); and patients who received chemotherapy previously may present acquired drug resistance. Thus prevalence of Multi-drug resistance is useful information on the implementation ofstandard chemotherapy regimens designed and recommended by WHO for tuberculosis patients who have or have not been treated previously.

Moreover, drug resistance rate can also serve as a useful parameter in the evaluation of quality of current and past chemotherapy programme (DOTS). Therefore, understanding the drug susceptibility patterns of *Mycobacteriumtuberculosis*is very crucial to treat patients, to decide health priorities, to allocate resources, to monitor the emergency of resistance for planning effective use of anti-TB drugs, to generate knowledge for health workers working in the study area as well as will serve as a preliminary information for health programmers to give special attention anddesign a package in the national TB control programme that addresses such areas where hundred to two hundred thousands of people are employed in huge farms for the production of crops.

4. OBJECTIVES

4.1 General Objective

-To assess the prevalence and associated risk factors of MDR-TB among smear positiveTB patientsin West Armachiho and Metema woredas of North Gondar.

4.2 Specific Objectives

- To determine the prevalence of MDR-TB in West Armachiho and Metema Woredas.
- To determine the prevalence of rifampicin resistant non-MDR-TB in West Armachiho and Metema Woredas.
- To assess risk factors associated with MDR-TB in West Armachiho and Metema Woredas.

5. MATERIALSANDMETHODS

5.1 Study area

The study was conducted in West Armachiho and Metema woredas, in North Gondar Zone of Amhara Region, North-west Ethiopia. West Armachiho woreda located about 210 kilometers far from Gondar, 390 Kilometers from the Regional Capital, Bahir Dar and 955 kilometers away from Addis Ababa, capital city of Ethiopia.Metema woreda is located about 180 kilometers far from Gondar, 360 Kilometers from the Regional Capital, Bahir Dar and 925 kilometers away from Addis Ababa in the North-west direction.The climatic condition of the woredas is mainly hot, and average annual temperature is estimated to be between 32^0C- 44^0C. The mean annual rainfall is about 1,521 mm. The Woredasare well known for Huge Agricultural Farms. Administratively,West-Armachiho Woreda is divided into 14 kebeles. According to the 2005 Ethiopian fiscal year (2012/13) report of North Gondar Health Department the population of the Woreda estimated to be 38,726.Every year up to 250,000 migrants comes to the woreda to be employed in the farms. The Woreda has three Health Centers 2 of them (Abderafi and Abirihajira Hcs) provides TB diagnosis and treatment and the other Gabla Hc provides only treatment. Abderafi and Abirihajira Health Centers are the only well-established referral Health Facilities in the woreda, together they provides Health care service to more than 22,000 peoples. Regarding Metema, The woreda is divided into 22 kebeles.According to the 2005 Ethiopian fiscal year (2012/13) report of North Gondar Health Department the population of the woreda estimated to be 142,569.The woreda has 6 Health Centers(Metema, Gendewuha, Shinfa, Kokit, Mesha, Meka) and Metema District hospitaland all of them provides TB diagnosis and treatment and together they provides Health care service to more than 450,000 peoples. And every year up to 500,000 migrants comes to the woredas to be employed in the farms.The woredas have public services like mobile telecommunication, postal services, and chlorinated water supply system, a12 hour's generator power in West Armachiho and 24 hrs Power in Metema.

5.2 Study design and period

A cross-sectionalstudy was conducted among confirmed smear positive tuberculosis patients who visited 9 health facilities in the woredas fromFebruaryto June 2014.

5.3 Population

5.3.1 Source population

The source population was all patients who had a compliant of tuberculosis sign and symptoms such as cough greater than two weeks, night sweating, weight loss more than 3 kilogram in a month and visited the health facilities during the study period.

5.3.2 Study population

All confirmed smear positive tuberculosis patients who were diagnosed in Abderafi, Abirihajira, Metema, Gendewuha health centers and Metema hospital, during the study period.

5.4 Sample size and sampling technique

The sample size for the study was determined by the following formula:

$$n = \frac{Z^2 pq}{d^2}$$

Where, n is the minimum sample size required;

Z= Is the Z score corresponding to α error of 5% (i.e., 1.96); or critical value at 95% certainty (1.96)

P= 5%, the prevalence of Multi Drug Resistance TB *=5%)*(24).

q=1-p

d= marginal error between the sample and the population (0.05). Accordingly, the calculated minimum sample size was 73. However, 124 study subjects were included in thestudy.

5.5 Sampling procedures

All consecutive smear positive TB patients diagnosed at the study sites during the study period were included in the study.

5.6 Inclusion criteria

All smear-positive pulmonary TB patientswere included in the study.

5.7 Exclusion criteria

- Smear positive TB cases who took treatment >1 month.

5.8Study variables

5.8.1 **Dependent variable**; MDR-TB

5.8.2 **Independent variables**;

Age, sex, residence, occupation, Income,educational status, HIV, smoking, TB contact history, diabetes, fasting, history of prison, BCG vaccination status, are some of the in-dependentvariables.

5.9 Data and sample collection

Data of socio-demographic and clinical variables from TB patients werecollected after careful examination of patient history for new and previous anti-tuberculosis treatment. A structured questionnaire was used to classify patients as ‘new’ and‘ previously treated’ TB cases and laboratory diagnosis of TB based on national guidelines for microscopic examination of TBwas performed :Three direct smears were prepared from three sputum specimens and stained using Ziehl-Neelsen and Florescent Microscopy techniques for microscopic examination of AFB. Once diagnosed, and confirmed of being smear positive tuberculosis patients, informed consent was obtained from study subjects and two morning sputum sample from 5 to 10ml was collected using standard sputum cup prior to starting anti-tuberculosis treatment. After collection sputum specimens were re-cupped tightly to prevent leakage of AFB and stored at -20 0c refrigerator till transported to University of Gondar Hospital and Bahir dar Regional Health Research LaboratoryCenter. For transporting specimens standard triple package transportation carrier was used.

Once the specimen reached University of Gondar Hospital, one sputum sample was used for detection of *Mycobacterium tuberculosis* complex and resistance to rifampicin using GeneXpert MTB/RIF- (fully-automated diagnostic molecular test, which simultaneously detects TB and rifampicin drug resistance and provides accurate results within two hours) and the other sputum sample was transported to Bahir dar Regional Laboratory Center for culture and drug susceptibility testing for INH and RIF using Lowenstein Jenson media when the strain detected as resistant to RIF by Gene**5.9.1 Sputum decontamination, isolation and identification**

Smear positive sputum samples werere-confirmed using Gene X-pert and decontamination and further homogenization was done according to Petroff's method, with equal volume of sodium hydroxide 4%, and centrifuged at 3000RPM for 20 minutes. Then the sediment was neutralized and washed. Sputum specimen inoculated onto LJ slant (prepared as per the dry powder manufacturer instruction).Incubated at 37oC and examined weekly. The result considered negative if no growth was observed after 8 weeks. All *mycobacterium* isolates were identified using standard biochemical methods and the presence of *M. tuberculosis*was confirmed by colonial morphology,positive niacin production and nitrate reduction tests.

5.9.2 Drug susceptibility testing (DST)

Sputum samples from all smear positive pulmonary tuberculosis cases were tested for drug susceptibility to rifampicin using Gene x-pert machine and those patients who were resistant to rifampicin tested for INH and RIFsusceptibility by solid culture method using LJ. Colonies of isolated MTB from positive cultures were prepared by scraping from LJ slant into 3 ml of sterile phosphate buffer saline (PBS) in sterile conical tube containing 4 glass beads. The tubes were vortexed vigorously for several minutes in order to break up the clumps. Then the larger particles were allowed to settle and the sediment was adjusted to a McFarland standard of 0.5. DST was performed against first line drugs(INH and RIF) using the indirect proportional method on LJ medium following standard procedures. Briefly, predetermined concentrations of drugs were incorporated into LJ medium before solidification. The slant was subsequently inoculated with the standardized prepared inoculum. Drugs at a concentration of INH 0.2mg/ml and RMP 40mg/ml wasused. The proportion method calculates the proportion of resistant bacilli present in a strain. Two appropriate dilution of the bacilli, 10^{-2} and 10^{-4}dilutions (undiluted = $10^{6}$to 10^{8} CFU/ml), were inoculated on drug-containing and drug-free media, in order to obtain countable colonies on both media. The ratio of number of colonies observed on the drug-containing media to drug-free medium indicates proportion of resistant bacilli present in the strain.

Below a proportion (critical proportion = 1%), the strain wasclassified as sensitive; otherwise classified as resistant. HIV test results of study subjects were collected from TB unit register at TB clinic of respective health facilities.

5.10 Quality control of laboratory methods

The reliability of the study findings was guaranteed by implementing Quality control (QC) measures throughout the whole process of the laboratory work. All materials, equipment and procedures were adequately controlled.

5.10.1 Quality control of X-pert

Each test includes a sample processing control and probe check.

Sample Processing Control (SPC)-Ensures the sample was correctly processed. The SPC contains non-infectious spores in the form of a dry spore cake that is included in each cartridge to verify adequate processing of MTB. The SPC verifies that lysis of MTB has occurred if the organisms were present and verifies that specimen processing was adequate. Additionally, this control detects specimen- associated inhibition of the real-time PCR assay. The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria. The test result will be "Invalid" if the SPC was not detected in a negative test.

Probe Check Control (PCC)-Before the start of the PCR reaction, the GeneXpert diagnosticsystem measures the fluorescence signal from the probes to monitor bead rehydration, reaction-tube filling, probe integrity and dye stability. Probe Check passes if it meets the assigned acceptance criteria.

5.10.2 Quality control of LJ Media

LJ culture media were tested for sterility by incubating for 48 hours at 37°c.Pre-analytical, analytical and post-analytical stages of quality assurance that was incorporated in standard operating procedures (SOPs) of the microbiology laboratory of Bahir dar Regional Health Research Laboratorywas strictly followed. Quality control of drug susceptibility testingwas performed by titrating the standard strain*M. tuberculosis* reference strain H37Rv (ATCC 27294)for each newly produced batch of drug susceptibility testingmedia.

5.11 Data quality control

To assure data quality, data collectors was trained for two days how to use pre designed form and 5% of the sample was pre-tested in non-selected study facility at University of GondarHospital. A constant monitoring process was framed as integral part of the data collection processes. The data collectors were supervised daily. Supervisor handled problems, which arose, and received and checked for completion of forms in order to clean up incorrect reporting and laboratory examination. The supervisor in turn communicated with the principal investigator each day to go through the completed forms and discuss problems. Therefore, there was a two-stage quality control process throughout the data collection. Quantitative data was double entered to check whether there was any inconsistency of data and avoided problems through the data entry processes.

5.12 Data analysis

Quantitative and Qualitative data were entered, cleared and analyzed using the statistical package SPSS version 20.0. After cleaning the data, descriptive statistics: percentages, means, medians, standard deviations and ranges were used to describe the finding for each variable. Bivariate logistic regression analysis was used to assess the association. P-value and 95% confidence interval were used to assess for statically significance.Additionally, tables and graphs were used for data presentation.

5.13 Ethical consideration

Before starting the data collection, ethical clearance was obtained from the School of Biomedical and Laboratory sciences, University of Gondar. Written permission was obtained from North Gondar Zone Health Department to West Armachiho and Metema Woreda Health Offices and respective health centers. The objectives of the study were explained to the participants in a local language (Amharic) and clarification was given. Written consent were obtained from each study participant and those patients who were found to be confirmed MDR-TB cases were linked to MDR-TB treatment initiation center at University of Gondar Hospital immediately for admission and treatment follow up. Information obtained in each course of the study was kept confidential.

5.14 Dissemination of results

The finding of this study will be officially distributed to all concerned bodies such as Amhara National Regional State Health Bureau, North Gondar Health Department, West Armachiho and Metema woredas Health office for interventions and Health service planning as well as to all health facilities whom data were collected. In addition, the study will be presented to the health staffs in health facility where the study is conducted. Moreover, the result will be presented at University of Gondar staffs and students' annual conference and other scientific conferences. A manuscript will be prepared and submitted for publication in an appropriate journal.

6. RESULTS

A total of 124 smear positive tuberculosis patients were included from five different heath centers in West Armachiho and Metema Woredas. The health centers were Abderafi, Abirihajira, Metema Yohannes, Gendewuha and Metema Hospital.

The proportion of smear positive tuberculosis cases in each health facilities were as follows: Abderafi 49(39.5%), Metema Hospital,34(29.8%), Abirihajira 21 (12.9%), Metema yohannes16 (12.9%) and Gendewuha 4 (3.2%).Majority, 80 (64.5%) of the study participants were males, the mean and median age of the study subjects were 32 and 29 years respectively. Their age ranges from16–75 years. Nearly half, 46 (48.1%) were in the age range of 26–35 year, while 37 (29.8%) were below 25 years old.

Of 124 study subjects, 66 (53.2%) were urban inhabitants and 40 (32.3%) were farmers. The majority, 116 (93.5%) of the study subjects were Christians by religion while the rest 8 (6.5%) were Muslims. More than half, 64 (51.6%) were illiterate (Table 1).

Table 1: Socio-demographic characteristics of study subjects in West Armachiho and Metema Woredas,NorthwestEthiopia February to June 2014.

Variable	**Number (%)**
Age group in year's	
<25	37(29.8%)
26-35	57(46%)
36-45	16(12.9%)
>46	14(11.3%)
Gender	
Male	80 (64.5%)
Female	44 (35.5%)
Resident	
Urban	66(53.2%)
Rural	58(46.2%)
Occupation:	
Farmer	40(32.3%)
House wife	28(22.6%
Government employee	4(3.8%)
Merchant	16(12.9%)
Daily laborer	19(15.3%)
Driver	5(4%)
Student	8(6.5%)

Income/ month:	
< 500 birr	39 (31.5 %)
500 birr - 999 birr	55 (44.4%)
≥ 1000 birr	25(20.2%)
No means of income	5(4.0%)
Religion:	
Christian	116(93.5%)
Muslim	8(6.5%)
Ethnicity:	
Amhara	102(82.3%)
Tigre	22(17.7%)
Educational Status:	
Illiterate	54(51.9%)
Primary School	31(29.8%)
Secondary School	16(15.4%)
Diploma	3(2.9%)
Marital Status:	
Married	51(49%)
Un married	38(36.5%)
Widowed	5(4.8%)
Divorced	10(9.6%)

Prevalence of Multi- drug resistant tuberculosis (MDR-TB)

Sputum samples of the 124 smear positive tuberculosis patients were tested for multi drug resistance tuberculosis by using Gene-Xpert MTB/RIF technique and conventional solid culture, The overall prevalence of MDR-TB was 7(5.7%) and prevalence of MDR-TB among new smear positive TB cases was2(2.3%) and among previously treated smear positive TB cases 5(13.9%). Outcome of the last treatment of the 5 previously treated smear positive Pulmonary TB cases which were confirmed MDR-TB, were 1 (20%) cured, 3(60%) were treatment failures and the other 1 (20%) was defaulter.

Of these confirmed MDR-TB subjects six were males and one was female(Figure1).Two of the confirmed MDR cases were co-infected with HIV while, the other five were sero-negative.The prevalence of HIV co-infected with MDR-TB was 28.6%.

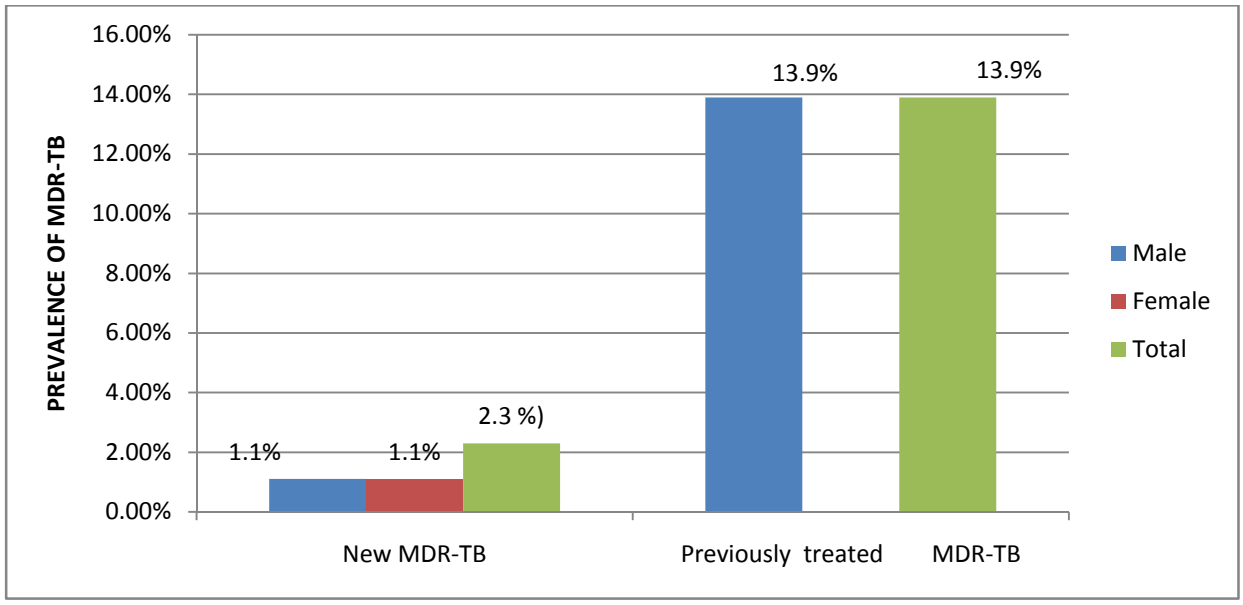
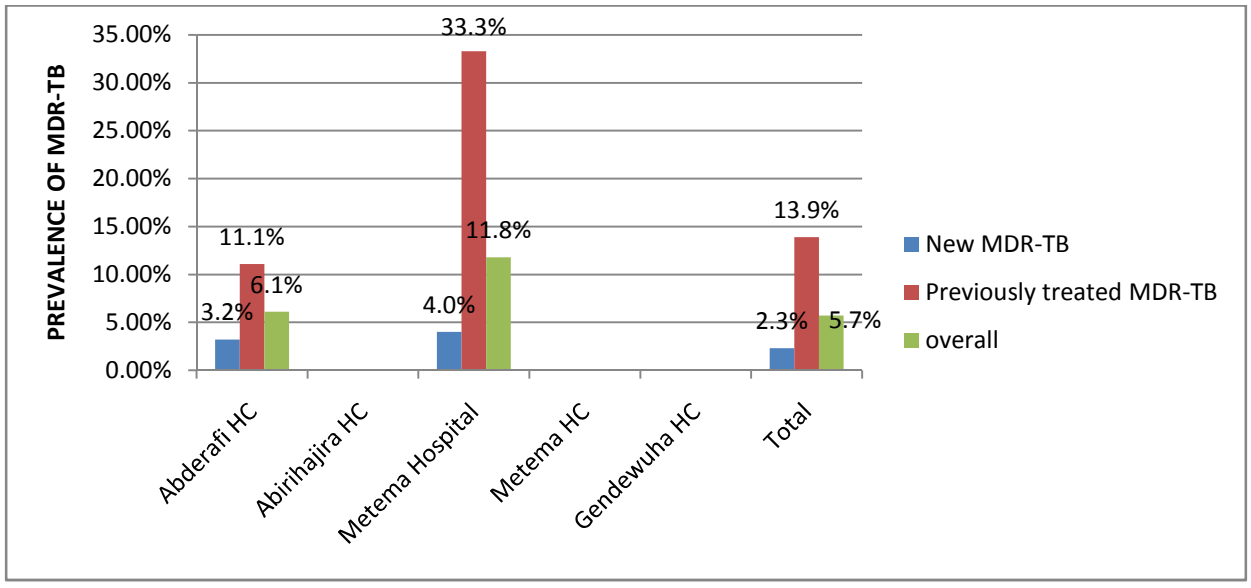


Figure 1: Gender distribution of Multi drug resistant tuberculosis

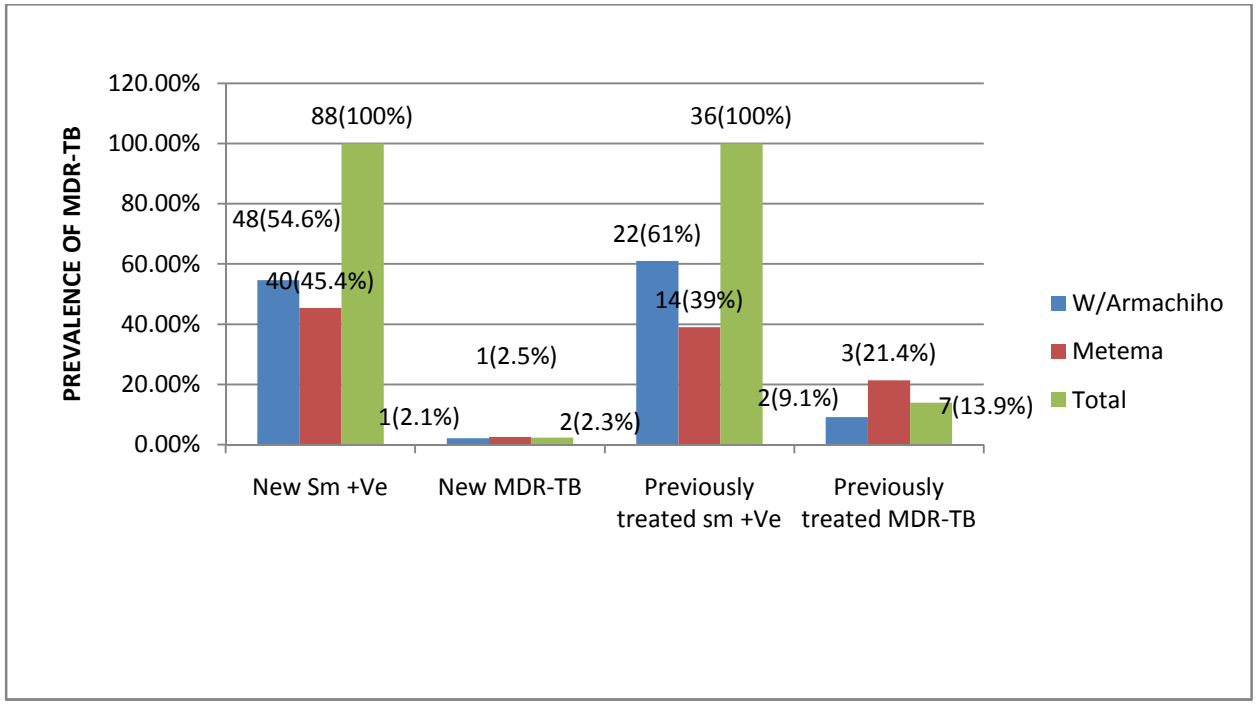
Among the five health facilities,MDR-TB cases were obtained from Abderafi health center and Metema District Hospital. Three MDR-TB cases (one from new smear positive study subjects and two from retreatment cases) were identified from Abderafi health center. The other four MDR-TB cases (one from new smear positive study subjects and three from retreatment cases) were identified from Metema hospital (Figure 2).



HC=Health Center

Figure 2: Prevalence of MDR-TB in Five Health Facilities of West Armachiho and Metema Woredas

Of 124 study subjects 70 study subjects were enrolled from West Armachiho woreda and the remaining 54 subjects were from Metema woreda.The prevalence of MDR-TB in West Armachiho woreda (4.3%)was slightly smaller than Metema woreda (7.4%) Figure3.



Sm+Ve=Smear Positive

Figure 3: Comparison of MDR-TB prevalence in West Armachiho and Metema woredas

Rifampicin resistant Non-MDR-TB

Sputum samples of 124 smear positive pulmonary TB cases were processed using GeneXpert MTB/RIF for detection of *Mycobacterium tuberculosis* and identification of rifampicin resistant strain. Of these only seven smear positive cases were found to be rifampicin resistant. Sputum samples of rifampicin resistant cases were further diagnosed with solid culture (Lowenstein Jenson) medium for growth of *Mycobacterium tuberculosis* and detection of isoniazid resistance as well as confirmation of rifampicin resistant strain. The result showed rifampicin resistant non-MDR-TB isolates were not observed from all rifampicin resistant isolates that were detected by GeneXpert MTB/RIF for drug susceptibility testing. All seven rifampicin resistant isolates were found to be MDR-TB cases.

Risk Factors for MDR-TB in West Armachiho and Metema woredas

Of the 124 study subjects, 88 (71%) were found to be new smear positive tuberculosis cases where as 36 (29.0%) were previously treated cases, and from those 36 previously treated study subjects 24 (66.7%) were treated for pulmonary tuberculosis once, whereas 11(30.5%) were treated twice at different time and place, the other 1 (2.8 %) where treated three times. Regarding the outcome of the previous treatment of study subjects 25 (69.4%) of them were cured/treatment completed, 6 (16.7%) were treatment failures, and 5 (13.9%) were defaulters.Of the 124 study subjects, 28 (22.6%) were found to be co-infected with HIV and the rest 96 (71.4%) were HIV negative. All study subjects did not have a history of taking anti-TB treatment from illegal drug dealers rather they took from public health facilities.

Of 124 smear-positive pulmonary TB patients, 35 (28.2%) have had a history of smoking whereas 89 (71.8%) were non-smokers, and of those smokers, 9 of them found to be smoking while they are taking anti-TB treatment. Eighty three (66.9%) of the study subjects have had a history of frequent contact with chronic coughers before they were infected with *Mycobacterium tuberculosis* whereas the other 41 (33.1%) were not. Regarding diabetic history of study subjects, 7 (5.6%) of them have had diabetics whereas the majority of them117 (94.4%) were non-diabetic. The majority of study subjects, 106 (85.5%) were without a history of imprisonment but 18 (14.5%) were imprisoned and from those imprisoned 8 (44.4%) were on custody for less than one month and the other 10 (55.6%) were on custody for more than one month. Vaccination status of the study subjects for BCG showed, 96 (77.4%) were not vaccinated but the rest 28 (22.6%) were vaccinated.

The relationship between individual exposure variables and MDR-TB status is shown in Table2. Association between potential exposure variables and MDR-TB were analyzed. Socio-demographic determinants such as age, sex.residence, occupation, income, religion, fasting, ethnicity, educational status, marital status, and factors such as contact history, history of imprisonment, number of rooms in the house, family number in the house hold, rooms for sleeping, number of windows and use of substances like cigarette smoking and other clinical characteristics such as, diabetes, history of previous anti-TB treatment, outcome of previous treatment,BCG vaccination,HIV status, history of taking illegal anti-TB treatment, were assessed

and all the variables that were considered important were entered to the binary logistic regression models and analysis showed there were significant association between MDR-TB and history of previous anti-TB treatment (OR:7, 95% CI=1.2-37.6, P=0.025). However, there were no significant association between other variables and MDR-TB. After adjustment for interactions among the independent variables with the binary regression model; analysis also showed there were no significant association between independent variables and prevalenceof MDR-TB (P>0.05) with each factors other thanprevious treatment history.

Table 2: Factors associated with the prevalence of MDR-TB among Pulmonary TB cases, West Armachiho and Metema woredas, Northwest Ethiopia,February to June 2014.

Variable	**Study subjects: N (%)**	**MDR-TB N (%)**	**Crude OR**	**P-Value**
Total	124(100%)	7 (5.7%)		
Age group in year's				
<25	37 (29.8%)	1(14.3%)	2.77 (0.16, 47.56)	0.483
26-35	57 (46%)	4 (57.1)	1.02 (0.11,9.90)	0.987
36-45	16 (12.9%)	1 (14.3%)	1.15 (0.07,20.34)	0.922
>46	14 (11.3%)	1 (14.3%)	1	
Gender				
Male	80 (64.5%)	6 (85.7%)	3.49 (0.41, 29.9 4)	0.255
Female	44 (35.5%)	1(14.3%)	1	
Resident				
Rural	58 (46.2%)	4 (57.1%)	1.56 (0.33, 7.26)	0.574
Urban	66 (53.2%)	3 (42.9%)	1	
Occupation:				
Farmer	40(32.3%)	4(57.1%)	3.15 (0.67, 14.79)	0.146
Non Farmer	88(67.7%	3(42.9%)	1	
Religion:				
Christian	116(93.5%)	7(100.0%)	1.16 (0.06,22.17)	0.919
Muslim	8(6.5%)	0(0%)	1	
Ethnicity:				
Amhara	102(82.3%)	4 (57.1%)	1	
Tigre	22(17.7%)	3 (42.9%)	3.87 (0.80,18.70)	0.092
Educational Status:				
Illiterate	54(51.9%)	2(28.6%)	5.17(0.41, 65.68)	0.206

Primary School	31(29.8%)	3(42.9%)	1.83(0.16,20.71)	0.624
Secondary School	16(15.4%)	1(14.3%)	2.67(0.14,49.76)	0.511
Diploma and Above	3(2.9%)	1(14.3%)	1	
Fasting				
Yes	76(61.3%)	3(43%)	0.45(0.10, 2.12)	0.313
No	48(38.7%)	4(57%)	1	
History of Smoking				
Yes	35(28.2%)	2(28.6%)	1.02(0.19, 5.51)	0.983
No	89(71.8%)	5(71.4%)	1	
BCG vaccination				
Yes	28(22.6%)	1(14.3%)	1	
No	96(77.4%)	6(85.7%)	1.80 (0.21, 15.61)	0.593
History of Previous Treatment				
Yes	36(29%)	5(71.4%)	6.94(1.28, 37.60)	0.025 *
No	88(71%)	2(28.6%)	1	
HIV status				
Yes	28(22.6%)	2(28.6%)	1.40(0.25, 7.64)	0.698
No	96(77.4%)	5 (71.4%)	1	
History of Prison				
Yes	18(14.5%)	1(14.3%)	0.98(0.11, 8.66)	0.986
No	106(85.5%)	6(85.7%)	1	
History of Contact to TB cases				
Yes	83(66.9%)	7(100%)	8.14(0. 45,146. 06	0.155
No	41(33.1%)	0(0%)	1	

N=number of subjects;OR=Odds Ratio

7. DISCUSSION

The present study was mainly aimed at determining the Prevalence of MDR-TB and risk factors for drug resistance tuberculosis among smear positive pulmonary cases in West Armachiho and Metema woredas, Northwest Ethiopia. The overall prevalence of MDR-TB among new and previously treated smear positive pulmonary tuberculosis cases were 5.7%. This finding is comparable to the rates reported in Northwest Ethiopia 5% (22), Nigeria 5.8% (32), and Karachi 5% (29), However, higher prevalence of MDR-TB were reported in Georgia 14.8% (23),India 20.4% (24),India 47.5%(28),Addis Ababa 43%(34),and Nigeria 76.4% (43).The higher level of prevalence of MDR-TB in Georgia, India and Addis Ababa compared to the present study might be due to the difference in the study subjects, As in the previous studies the majority of study subjects included were MDR-TB suspects whom they were referred from different facilities for confirmation of MDR-TB, drug susceptibility test and treatment due to failure of TB patients to first line anti-tuberculosis treatment.On the contrary the present study showed a higher prevalence compared to the findings in India1.1% (25),Gujarat2.4% (27),Pakistan1.8% (30), uMzinyathiDistrict, KwaZulu-Natal state, South Africa2.3% (31),Nationwide anti-TB drug resistance survey conducted in Ethiopia by EHNRI/ FMOH in 20052.0% (2,21),Addis Ababa0.6% (33) and Jimma1.5% (46).the reason for the observed difference might be the difference in the study periods as the previous studies were conducted in a relatively low prevalence of MDR-TB era.

The prevalence of MDR-TB among new smear positive pulmonary TB cases in this study was 2.3% comparable to the studies conducted in Gujarat2.4% (27),Karachi, Pakistan2.3% (29), and Addis Ababa2.3% (35).However, it is lower than from findings in Georgia, Eastern Europe 6.8% (23), and Swaziland 7.7% (44); the lower prevalence in this study might be due to MDR prevalence in the general populationis very high in those countries compared to Ethiopia. However, this study reported a higher prevalence of MDR-TB from new cases (2.3%) compared to the findings of the Nationwide anti-TB drug resistance survey in Ethiopia 1.6% (2,21), India1.1% (25), and Jimma1.5% (46).The reason for the higher MDR-TB prevalence among new cases in the present study might be large number of people to work in huge farms for the production of crops in the study area share the same house or camp for sheltering and mostly crowded and it also reflects the wider spread of MDR-TB in the area and the fact that sufficient

control measures are not taken to prevent the development and the transmission of drug-resistant TB in study areas.

Of the 36 previously treated smear positive tuberculosis cases included in this study 5(13.9%) were found to be MDR-TB, this finding is lower than other studies in Georgia27.4% (23),India20.4% (24), Korea27.2% (26), Karachi17.9% (29) and in Addis Ababa71.4%(35).The lower prevalence in the present study might be due to the difference in study subjects,as the previous studies included only TB patients referred for culture and DSTfor confirmation of MDR-TB and treatment. However the present study showed a higher prevalence compared to the findings of two studies in Ethiopia: the Nationwide anti-TB drug resistance survey 11.8%(21) and First- and second-line anti-tuberculosis drug resistance in Northwest Ethiopia 10.9%(22).The higher prevalence of the present study compared to the previous two studies in Ethiopia might be due to the fact that high proportion of previously treated in the study areas i.e. 29%of the smear positive cases were found to be previously treated unlike the prevalence survey finding 8.6% (21),and First-line and second-line anti-tuberculosis drug resistance in Northwest Ethiopia17.7% (22).Moreover this finding may show poor implementation of DOTS in the study area; though, all the five facilities are TB treatment health facilities, most of study subjects 59(47.6%) were either farmers or daily labors and this study subjects spent their time working in huge farms where there is no nearby clinic or temporary DOTS center in the camp that provides DOTS. Implementation of poor quality of DOTs might have contributed to the emergence of TB treatment failures, defaulters and relapses which leads to high prevalence of MDR-TB in previously treated patients in the study areas.

MDR-TB among previously treated patients had higher prevalence than among new subjects, this finding agrees with findings of other studies in Asian countries: Korea, 27.2% among previously treated and 3.9% among new subjects (26), Karachi, 17.9%among previously treated and 2.3% among new cases (29) , Georgia, 14.8% among previously treated and 2.3% among new (23),and other studies from Ethiopia: Nationwide anti-TB drug resistance survey conducted by EHNRI/ FMOH, 11.8% among previously treated and 1.6 % among new (21), First- and second-line anti-tuberculosis drug resistance in Northwest Ethiopia, 10.9%among previously treated and 3.7% among new cases (22).

The prevalence of MDR-TB in the two districts is quite different compared to each other; from 70 smear positive pulmonary TB study subjects enrolled in West Armachiho woreda 3 of them were found to be 4.3% (2.1% among new subjects and 9.1% among previously treated) which is lower than the findings in Metema 7.4%,(5% among new subjects and 21.4% among previously treated) the reason for the higher prevalence in Metema woreda might be due to unequal recruitment of study subjects in both woredas and West Armachiho woreda contributes 70 subjects whereas Metema woreda only 54.

Of the 7 Rifampicin resistant strains identified by using X-pert MTB/RIF (GeneXpert diagnostic system) none of them were isoniazidsensitive. This finding is different from other studies in India1.1% (25), and Addis Ababa 1.2% (33). The reason might be due to the relatively low number of rifampicin resistant strains identification in the present study.

In the present study association between potential exposure variables and MDR-TB were analyzed using logistic regression model. History of previous treatment was the only significantly associated risk factor with MDR-TB (OR:7, 95%CI=1.28-37.6,P=0.02).This finding agrees with findings of other studies such as international drug resistance survey(41), Europe; Georgia(23), four European Union countries: France, Germany, Italy, and Spain (38), Asian countries: Korea(26),Karachi(29),and India(40),also with other African countries such as Nigeria (43), and Swaziland (44).Moreover, this study agrees with other findings in Northwest Ethiopia(22),and Addis Ababa(47), and in this study previously treated patients have had seven times at risk of developing MDR-TB than new smear positive pulmonary cases.

In the current study, HIV status had no significant association with MDR-TB. This finding agrees with the finding in other studies in Ethiopia: the nationwide anti-TB drug resistance survey (21), First- and second-line anti-tuberculosis drug resistance in Northwest Ethiopia (22), Addis Ababa (35), and Jimma (46), had come up in their respective finding that HIV status was not the risk factor for the development and emergence of MDR-TB.However, other studies from African countries Nigeria (32) and Swaziland (44) contradicted this finding and concluded HIV was significantly associated with MDR-TB. The possible explanation for HIV infected study subjects were not at risk for the development of MDR-TB than sero-negative patients in the present study might be, being HIV positive might lead the study subject to be infected with primary tuberculosis due to immunosuppression and TB is the major opportunistic disease that

might occur throughout the life time of HIV positive subjects.Hence HIV didn't specifically lead*Mycobacterium tuberculosis* strain to undergo spontaneous mutation so that it would promote DR-TB.

The present finding shows no significant association of age with MDR-TB. This study is in agreement with studies from four EuropeanUnion countries: France, Germany, Italy, and Spain(38), India (40), Nigeria (43),and other parts of Ethiopia; Jimma (46), and Addis Ababa (47). However, other studies in Honk Kong(36), Swaziland(44), and Bangladesh (45) reported that younger age group was at risk for the emergence and development of MDR-TB. On the contrary: study in Spain (39),showed that getting old was the risk factor for MDR-TB compared to younger age group.In the present study Gender was also not significantly associated with MDR-TB, unlike the findings of other studies in Georgia (23),Karachi(29),and Swaziland (44), which reported being female was the risk for MDR-TB. However, similar findings compared with the present study were reported in Korea(26),Nigeria(32,43),India(40),Bangladesh(45),Jimma(46),and Addis Ababa(47).A report in Hong Kong(36), showed non-permanent resident and frequent travel was significantly associated with MDR-TB.However, this study did not show any significance difference. The reason for the insignificant association of socio-economic characteristics such as age, sex, resident in this study might be the small number of MDR-TB cases identified in this study.

8. STRENGTH

The use of a fully-automated diagnostic molecular test for diagnosing Rifampicin resistant *Mycobacterium tuberculosis* complex (GeneXpert MTB/RIF) in this study could be considered as the strength. Moreover, all the study subjects identified as MDR-TB was successfully linked to the MDR-TB treatment initiation center of the University of Gondar teaching hospital.

9.LIMITATION

The present study could not be without limitations and the main limitation that occurred was the small number of MDR-TB cases resulted difficulty in applications of statistical packages to assess risk factors associated with MDR-TB. Only accessible facilities in terms of transportation and facilities with relatively higher number of smear positive TB cases in West Armachiho and Metema woredas were included in the study.

10. CONCLUSION

On this particular study, a considerably high overall prevalence of MDR-TB among new and previously treated cases 5.7%was founded. The prevalence of MDR TB among retreatment cases was greater than the new cases 13.9% and 2.3%, respectively. History of previous anti TB treatment were the only statistically significant risk factor for MDR TB. All seven rifampicin resistant strains were also isoniazid resistant (rifampicin resistant non-MDR-TB cases were not identified).

11. RECOMMENDATION

Based on the study findings the following recommendations are forwarded.

- Establishment of seasonal DOTS centers and diagnostic laboratory facilities should be considered, as there are huge farms and continues flow of people for work.
- Improved infection control measures need to be strengthened and implemented in the study area and broadly in the North Gondar to reduce the risk of DR-TB transmission in the community.
- Daily laboures who had been working in these woredas should be screened for tuburclosis before they want back to home.
- Establishing MDR-TB initiation as well as diagnostic centers to cope up with considerably high prevalence of MDR-TB patients in the study area.
- Regular monitoring mechanism of proper implementation of DOTS should be established to increase treatment success rate thereby to reduce treatment failures, defaulters.
- Further studies should be conducted in the study areas with special focus on the determinants for the considerably high prevalence of MDR-TB in the area.
- Provision of rapid diagnostic tools for the identification of drug resistance TB in the referral health facilities, so as to increase case detection and identification of MDR-TB.

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13. ANNEXES

ANNEX 1. Questionnaire (English Version)

Prevalence of MDR-TB and associated Risk Factors inWest Armachiho and Metema woredas of North Gondar Zone, North West Ethiopia Questionnaire

Case Number: _____Lab.No_____

Diagnostic center_____Date of sputum collection: _____Time of collection_____

A. Socio-demographic data of the patient

1.Age: _____ (Exact number)

2. Sex:

1. Male 2.Female

3. Resident

1.Urban 2. Rural

4. Occupation:

1. Farmer 2. House wife

3. Government employee 4. Merchant

5. Daily laborer6. Driver

7. Other specify_____

5. Income/ month:

a. < 500 birr

b. 500 birr - 999 birr

c. ≥ 1000 birr

d. No means of income

e. Other specify

6. Religion:

1. Christian

2. Muslim

3. Other (specify) _____

7. Do you fast on regular basis?

1. Yes

2. No

8. Ethnicity:

1. Amhara
2. Tigre
3. Other (specify)_____

9. Educational Status:

1. Illiterate
2. Primary School
3. Secondary School
4. Diploma
5. Degree and above

10. Marital Status:

1. Married
2. Un married
3. Widowed
4. Divorced

B. Risk Factors

B1**.** Do you have a history of cigarette Smoking?

1. Yes
2. No

B2.Are you Smoking now?

1. Yes
2. No

B3. Do you have history of contact to a person with chronic cough?

1. Yes
2. No

B4.Do you have diabetes?

1. Yes
2. No

B5. Is the patient previously treated for TB?

1. Yes

2. No

B6.If the answer in B5 is yes,

B6 .1.How long have you been treated for TB before? (in months)----------

B6.2.How frequent have you been treated for TB before?

1. Once

2. Twice

3. Three times

B7. Outcome of the last treatment according to the patient:-

1. Cured/ Treatment completed

2. Failed

3. Defaulted

4. Unknown

B8. History of prison stay

1.Yes

2.No

B9. If the answer to B8. Is yes for how long(weeks)_____

B.10.How many rooms do you have?_____

B.11.Total number of family members? _____

B.12. How many rooms in this household are used for sleeping? _____

B.13.How many windows in the household are there? _____

B.14.On average how many people are sleeping in one bedroom in your household?

_____

B.15.BCG vaccination status

B.15.1 BCG scar: 1.Present 2.Absent

B.15.2 BCG vaccination card: 1.Present 2.Absent

B.16 HIV Status 1.Positive 2.Negative

B.17.History of Illegal drug use 1.Yes 2.No

Name of data collector_____Signature_____Date_____

ANNEX2.Amharic Questionnaire

በሰሜንጎንደርዞንበምዕራብአርማጭሆእናመተማወረዳዎችመድህኒትየተለማመደቲቢእናየችግሩተያያዥኢጋላጭመንስኤዎችለማ ጥናትየተዘጋጀመጠይቅ

1. የግለሰቡ ማህበራዊ ና ሥነ-ህዝባዊ መረጃዎችን ለማጥናት በተመለከተ

ተ.ቁ	*ጥያቄዎች*	*አማራጭመልሶች*	*ኮድ*	*ይለፍ*
1	ዕድሜዎ ስንትነዉ?	..... ዓመት		
2	ፆታ	1.ወንድ 2.ሴት		
3	የመኖሪያቦታዎየትነዉ?	1.ከተማ 2.ገጠር		
4	መተዳደሪያዎምንድንነዉ?	1. ገበሬ 2. የቤትእመቤት 3. ተማሪ 4. ነጋዴ 5. የመንግስትሰራተኛ 6. ሌላካለይገለጽ		
5	የወርገቢዎምንያህልነው?	1. ከ500 ብርበታች 2. ከ500 እስከ 999 ብር 3. ከ1000 ብርበላይ 4. ምንምየገቢምንጭየሌለው 5. ሌላካለይጠቀስ.....		
6	የምንሀይማኖትተከታይነዎት?	1. ክርስቲያን 2. ሙስሊም 3. ሌላካለይገለጽ----------		
7	አዘውትረውይፆማሉ?	1. አዎ 2. አይደለም		
8	የምንብሄርአባልነዎት?	1. አማራ 2.ትግሬ 3.ሌላ ከሆነይጠቀስ		
9	የትምህርትደረጃ	1. ያልተማረ 2. 1ኛ ደረጃያጠናቀቀ 3. 10ኛ /12ኛ/ ያጠናቀቀ 4. ዲፕሎማናከዚያበላይ		
10	የጋብቻሁኔታዎምንድንነው?	1. ያገባ 2. ያላገባ 3. የሞተበት 4. የተፋቱ		
2.የችግሩ ተያያዥኢጋላጭመንስኤዎች				
2.1	ከአሁንበፊትሲጋራያጨሱነበር?	1. አዎ2. አላጨስም		
2.2	አሁንስያጨሳሉ?	1. አዎ2. አላጨስም		
2.3	ለብዙጊዜሳልከሚስልሰውጋርግንኙነትነበረ	1. አዎ2. የለም		

	ዎት?			
2.4	የስኳርህመምተኛነዎት?	1. አዎን 2. አይደለሁም		
2.5	ከአሁንበፊትበቲቢበሽታታክመዉያዉቃሉ?	1. አዎ 2. አይደለም		
2.6.1	ለጥያቄቁጥር 2.5 መልስዎ “አዎ” ከሆነለምንያህልጊዜተከታትለዋል? (በ ወራት)	----------		
2.6.2	ለጥያቄቁጥር 2.5 መልስዎ “አዎ” ከሆነ ለምንያህልጊዜ ደጋግመዉ ተከታትለዋል?	1. አንድጊዜ 2. ሁለትጊዜ 3. ከሁለትጊዜበላይ		
2.7	በመጨረሻውየህክምናክትትልጊዜየበሽተኛ ውውጤትምንነበር?	1. የዳነወይምህክምናየጨረሰ 2. ህክምናዉ የ መከነ 3. ህክምናዉየተቋረጠ 4. ያልታወቀ		
2.8	የህግታራሚሁነዉማረሚያቤትገብተዉያዉ ቃሉ?	1. አዎ2. አይደለም		
2.9	ለጥያቄቁጥር 2.8 መልስዎ “አዎ” ከሆነለምንያህልጊዜ (በ ሳምንታት)			
2.9	መኖሪያቤትዎ ምንያህል ክፍሎቸ አሉት?			
2.10	የቤተሰብዎአባላት ምንያህል ናቸዉ?			
2.11	በመኖሪያቤትዎ ምንያህል ክፍሎችለመኝታአገልግሎትይዉላሉ?			
2.12	በመኖሪያቤትዎ ምንያህል መስኮቶችአሉ?			
2.13	በመኖሪያቤትዎበ አንድአልጋምንያህል ሰዎችይተኛሉ?			
2.14	ለቲቢበሺታ መከላከያ ክትባትሁኔተ			
2.14.1	ለቲቢበሺታ መከላከያ ክትባት መዉሰዳቸዉን የሚያረጋግጥጠባሳምልክት?	1. አዎ 2. የለም		
2.14.2	ለቲቢበሺታ መከላከያ ክትባት መዉሰዳቸዉን የሚያረጋግጥካርድ?	1. አዎ 2. የለም		
2.15	ኤችአይቪውጤት	1.ፖዘቲቭ2.ኒጋቲቭ		
2.16	ከ ጤና ተቁዋም ዉጭ የቲቢ መድሃኒት ወስደዉያዉቃሉ?	1. አዎ2. አላዉቅም		

ይህንንመረጃክቡርጊዜዎን ሰዉተዉ ስለሰጡንበጣምእናመሰግናለን!!

የጠያቂውስም……የተቆጣጣሪውስም……

ቀን …… ቀን ……

ፊርማ …… ፊርማ ……

ANNEX 3: Information and Consent form

Title of the Research Project: Prevalence of MDR-TB and associated Risk Factors in West Armachiho and Metema Woredas of North Gondar, North West Ethiopia

Name of Investigator: Feleke Mekonnen Demeke (BSc, MSc candidate)

Name of the Organization: University of Gondar, School of Biomedical and Laboratory science, Department of Medical Microbiology

Introduction

You are invited to participate as study subject in a research conducted by MSc candidate, from University of Gondar. Your participation is voluntarily. The research teams include one principal investigator, sample collectors, three advisors from university of Gondar. Please take as much time as you need to read or listen the information sheet.

Purpose of the Research Project

We are asking you to take part in this study because we are trying to learn more about the prevalence of MDR-TB in West Armachiho and Metema woredas and risk factors associated for the exposure to this catastrophic disease. This research is designed for effective prevention and control measure.

Procedure

In order to perform the indicated study at Gondar university hospital you are invited to take part in this project. If you are willing to participate, you need to understand the purpose of the study and give your consent. The required sputum sample will be collected by Laboratory personnel who are currently working in the health center. Then, you are requested to give your consent to the sample collector.

Potential Risks and Discomforts

There are no anticipated risks to your participation. You are simply requested to take some time and give productive sputum sample. During collection of sputum you should collect sputum in places where there is well ventilatilation and no other persons at time of collection not to

transmit the disease to others but personally there is no any procedure or discomfort that will affect your health.

Potential benefits to subjects and/or to the society

Based on the diagnosis result you will be treated accordingly. On the other hand, the result of the study will be beneficial to design effective prevention and control measure for MDR-TB. Hence, you are indirectly benefiting other patients and the society in this respect.

Compensation for participation

You will not receive any payment for your participation in this research study.

Confidentiality

There is no sensitive issue that you will be asked related with your social desirability but any information that is obtained in connection with this study and that can be identified with you will remain confidential. The information collected about you will be coded using numbers.

Participation and withdrawal

You can choose whether to be a part of this study or not. You may withdrawal at any time without consequences of any kind. You may also refuse to give any sample.

Person to contact

If you have any question you can contact any of the following (Investigator and Advisors) and you may ask at any time you want.

FELEKE MEKONNEN (BSc),Cell phone: +251- 09 18 701291/ 0913 863665

Dr. BELAY TESSEMA (PhD.), Cell phone: +251- 09 19306918

Dr. FELEKE MOGES (PhD.), Cell phone: +251- 09 18 778160

Mr. ASCHALEW GELAW (MSc.), Cell phone: +251- 09 18 711787

Patient consent form

I the undersigned patient with smear positive Tuberculosis case have been well informed about the objective of the study entitled "Prevalence of Multi Drug Resistance Tuberculosis and Associated Risk Factors in West Armachiho and Metema Woredas of North Gondar, North West Ethiopia". I am also told that all information obtained at any course of the study is to be kept confidential. Moreover, i have also been well informed of my right to keep hold of, decline to cooperate and drop out of the study if i want and none of my actions will have any bearing at all on my overall health care and hospital access.

Therefore, with full understanding of the situations i agree to give the entire necessary information and sputum samples for laboratory analysis.

Name_____Signature_____Date_____

For families or attendants of patients unable to respond

I_____ parent/guardian/attendant, after being fully Informed about the purpose of this study, hereby give my consent on the patient's participation in this study. I understand that my child free to withdraw at any time without penalty or loss of benefits.

Signature_____Date_____

ANNEX4.የጥናቱ ማብራራያ / information sheet/

ı. የጥናቱርዕስ

መድሃኒት የተለማመደቲቢበሽታስርጭትእናተያያዠለከፋአደጋሊያጋልጡየሚችሉመንሰስኤምክንያቶቸንማወቅ

2. ጥናቱንየሚያካሒውሰውፈለቀመኮንንደመቀ

3. የትምህርትደረጃየሁለተኛዲግሪተማሪ/Msc student /

4. የመስሪያቤቱስም

የጎንደርዩኒቨርስቲህክምናፋኩሊቲየባዬሚዲካልእናላቦራቶሪሳይንስትምህርትቤትየማይክሮባዬሎጂትምህርትክፍል

5. ለጥናቱተሳታፊዎችየተሰጠማብራሪያ

5.ı. መግቢያ

በመጀመሪያእርዕስዎበዚህጥናትውስጥተሳታፊእንዲሆኑሲጠየቁተሳታፊየሚሆኑትፈቃደኛከሆኑብቻነው፡፡ይህንንጥናትየሚያካሒዱትሰዎችአንድዋ ና ተመራማሪ ሶስት የጥናቱአማካሪዎችእናናሙናሰብሳቢዎቸንያካተተነው፡፡

5.2. የጥናቱዓላማ

መድሃኒት የተለማመደቲቢበሽታስርጭትእናተያያዠለከፋአደጋሊያጋልጡየሚችሉመንሰስኤምክንያቶቸንማወቅ፡፡

5.3. ለጥናቱየሚያስፈልግናሙናአወሳሰድ

ይህንጥናትለማካሔድየዕርሱንፈቃደኛነትዎንማግኘትአስፈላጊነው፡፡የጥናቱንአላማናጥቅምበሚገባተረድተውለመሳተፍፍላጎትካለውት

ሀ. ናሙናለሚወስደውሰውወይምዋናተመራማሪለሚጠይቅዎትጥያቄተገቢውንመልስይሰጣሉ፡፡

ለ. ለምርምርየሚያስፈልገውንናሙናይሰጣሉ፡፡

5.4. ጥናቱየሚያስገኘውጥቅም

የምርምሩውጤት መድሃኒት የተለማመደቲቢበሽታስርጭትለመግታትናለመቆጣጠርይረዳል፡፡

5.5. በዚህጥናትውስጥበመሳተፍሊከሰቱየሚችሉስጋቶችናየምቾትመጓደሎች

ለጥናቱበሚወስደውናሙናምክንያትሊከሰትብዎየሚችልየተለየችግርእናየሚያሰጋምንምአይነትነገርየለም

5.6. ምስጥርአጠባባቅ
ለዚህጥናትከርስዎየምንወስደዉመረጃወይምናሙናየርስዎንማህበራዊተቀባይነትስጋትላይእይጥልም፡፡ይህቢሆንከርስዎየተገኘማንኛውምመረጃበሚስጥርእንደሚያዝለማረጋገጥእወዳለሁ፡፡

5.7. በጥናቱየሚሳተፉወይእራስንየማግለልመብት

በጥናቱውስጥመግባትባለመፈልግዎተሳታፊሆነውማቋረጥሲፈልጉበጤናነክእንክብካቤላይምንምአይነትተጽኖአይደረግም፡፡ እንዲሁምተሳታፊበመሆንዎየተለየእንክብካቤአያገኙም፡፡

ተጨማሪመረጃከፈለጉዋናውንተመራማሪወይምአማካሪዎችማነጋገርይችላሉ::

የተመራማሪዉየሞባይል ስልክቁጥር 0918 70 12 91/0918863665

የአማካሪዎችስልክቁጥር

ዶ/ር በላይ ተሰማ የሞባይል ስልክቁጥር+251- 09 19306918

ዶ/ር ፈለቀ ሞገስየሞባይል ስልክቁጥር+251- 09 18 778160

አቶ አስቻለዉ ገላዉ የሞባይል ስልክቁጥር+251- 09 18 711787

በምርምሩለመሳተፍየስምምነትመግለጫ

እኔከዚህበታችስሜየተጠቀሰውናየፈረምኩትየሳንባነቀርሳህመምተኛ "መድሃኒት የተለማመደቲቢበሽታ ስርጭት እናተያያዥ ለ ከፋአደጋሊያጋልጡየሚችሉመንሰስኤምክንያቶቸንለማወቅ"
በጥናቱባልሳተፍምሆነአቋርጬብወጣከጤናተቋሙበማገኘውየህክምናአገልግሎትምንምአይነትችግርእንደማደርስብኝተነግሮኛ ል::በመሆኑምየአክታናሙናምሆነለምጠየቀዉመጠይቅመልስለመስጠትሙሉፈቃደኛመሆኔንእገልጻሁ::

ስም----------ፊርማ _____ ቀን: -----/-----/-----

ለሕፃናትና ሀሳባቸውን መግለፅ ለማይችሉ አዋቂዎች አስታማሚዎች

እኔ _____ የበሽተኛው አስታማሚ ስሆን የዚህን ጥናት ዓላማ በውል በመገንዘብ በሽተኛው በጥናቱ እንዲሳተፍ የምስማማ መሆኔን በፊርማዬ አረጋግጣለሁ::

ፊርማ _____ ቀን: -----/-----/-----

ANNEX 5**:** ***Principle and Procedure of LJ medium***

Lowenstein–Jensen (LJ) drug-containing media were used for drug susceptibility testing of *M. tuberculosis* according to the proportion method.

Equipment and materials

The equipment and materials needed were:

- Balance (sensitivity 0.01 g)
- Spoons or spatulas
- Weighing paper
- Autoclave
- Inspissator and racks to hold slopes in correct position
- pH meter
- Simple dispenser device – either an automatic pipetting aid or a mechanical pipetting aid with thumb wheel or rubber teats
- Appropriate sterile glassware and sufficient sterile screw-capped tubes for media dispensing

Note: *Because of the risk of infection if glass tubes break, thick-walled, shock-resistant glass tubes or vials were used.*

Material for homogenization of egg mass (5-mm glass beads for manual shaking, a plate with magnetic stirrer, or a sterile low-speed blender with sterile glass/stainless steel bowl).

Thick walled conical flask, 2500 ml, Bunsen burner, Forceps, Funnel with sterile gauze, for filtration of homogenized egg preparation, Water-bath, Brown bottles, Vortex mixer

General equipment and glassware of a laboratory for media preparation

Small desicants, device for drug storage in dry conditions

Silica gel, Refrigerator

Reagents and solutions

Drug-containing media

Drugs are added to the still-liquid LJ medium before inspissations. Final drug concentrations were indicated in Table below.

Each test-tube were labelled with the following information:

- Date of preparation, name, and concentration of drug.

Final concentrations of drugs for quality control and for test strains

Test drugs		**Solvent**		**Final mass concentration in culture medium (mg/litre)**	
Designation	**Abbre**	**Solvent**	**Dilution**	**For quality control H37Rv**	**Critical conc.**
Isoniazid	INH	Sterile dw	Sterile dw	0.025•0.05•0.10	0.2
Rifampicin	RMP[a]	DMSO	dw	2.5•5.0•10.0	40.0

NB- forDrug susceptibility testing inproportion method - Drugs were stored indesiccators according to the manufacturers' instructions. The amount weighed were calculated according to the activity (potency) declared by the manufacturer and the water content.

Preparation of Drugs for DST

A standard batch (1620 ml) of LJ basic culture medium wasPrepared according to the SOP "Preparation of plain egg-based media". If multiple growth controls were included in the DST schema, the volume of this standard batch must be increased enlarged accordingly.

- <u>Isoniazid (INH)</u>

  For dry and pure INH, the correction factor was 1.

  Solution I: 10.0 mg INH dissolved in 50 ml sterile water (200 µg/ml)
  Solution II: 2.5 ml Solution I, made up to 25 ml with sterile water (20 µg/ml)
  Solution III: 5.0 ml Solution II, made up to 10 ml with sterile water (10 µg/ml)

Stock solution I (200 µg/ml)aliquated into sterile cryovials and stored frozen at -20 °C.

Final drug concentration in media (µg/ml):

	0. 2 µg/ml	0.1 µg/ml	0.05 µg/ml	0.025 µg/ml
Medium (ml)	198	19.8	19.8	19.8
Solution II (ml)	2	–	–	–
Solution III (ml)	–	0.2	0.10	0.05[a]
Water (ml)	–	–	0.10	0.15[a]
Final volume (ml)	200	20	20	20

[a]Graduated glass pipettes or single-channel adjustable microlitre-pipettes with sterile tips were used for accurate delivery.

- Rifampicin (RMP)

The correction factor was usually 1 for pure RMP or 1.03 for the sodium salt:

Solution I:

Factor 1: 40.0 mg RMP, dissolved in 10.0 ml DMSO (4000 µg/ml)

Factor 1.03*:* 41.2 mg RMP, dissolved in 10.0 ml sterile water (4000 µg/ml)

Solution II: 2.5 ml Solution I, made up to 10.0 ml with sterile water (1000 µg/ml)

Final concentration in drug media (µg/ml):

	40 µg/ml	10 µg/ml	5 µg/ml	2.5 µg/ml
Media (ml)	198	19.8	19.8	19.8
Solution I (ml)	2	–	–	–
Solution II (ml)	–	0.2	0.10	0.05[a]
Water (ml)	–	–	0.10	0.15[a]
Final volume (ml)	200	20	20	20

[a]Graduated glass pipettes or single-channel adjustable microlitre-pipettes with sterile tips were used for accurate delivery.

ANNEX 6: Principle and Procedure GeneXpert

The GeneXpert Dx System integrates and automates sample processing, nucleic acid amplification, and detection of the target sequences in simple or complex samples using real-time PCR and reverse transcriptase PCR. The system consists of an instrument, personal computer, barcode scanner, and preloaded software for running tests on collected samples and viewing theresults. The system requires the use of single-use disposable GeneXpert cartridges that hold the PCR reagents and host the PCR process. Because the cartridges are self-contained, cross-contamination between samples is eliminated. Xpert MTB/RIF includes reagents for the detection of tuberculosis and RIF's resistance as well as a sample processing control (SPC) to control for adequate processing of the target bacteria and to monitor the presence of inhibitor(s) in the PCR reaction. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability. The primers in the Xpert MTB/RIF assay amplify a portion of the *rpoB* gene containing the 81 base pair "core" region. The probes were able to differentiate between the conserved wild-type sequence and mutations in the core region that were associated with RIF's resistance.

Specimen Collection and Transport

- A minimum of 1 mL of sputum per specimen were Collected.
- Specimens were held at 2–8 °C prior to processing whenever possible. However, if necessary the specimens'could notprocessed, specimens were stored at a maximum of 35 °C for 3 days and at 4 °C for days 4-10.

1. Thepatient's were advised to Rinse mouth twice with water.

2. Thepatient's were advised to unscrew the lid on the sputum collection container.

3. Thepatient's were advised inhale deeply, cough vigorously, and expectorate the material into the sterile screw-capped specimen collection container. Avoid spills or soiling the outside of the container.

4. Secure the lid on the collection device.

5. Specimens were held at 2–8 °C whenever possible including during transport to the laboratory if not they were stored at -4°C.

Procedure for Sputum Sediments

Specimens with obvious food particles or other solid particulates were not accepted.

Volume Requirements—Sputum sediments prepared according to the method of Kent and Kubica8 and re-suspended in 67mM Phosphate/H2O buffer) can be tested using Xpert MTB/RIF. Once the resuspension was prepared for standard laboratory smear or culture tests, at least 0.5 mL of resuspended sediment were ensured available to run Xpert MTB/RIF.

1. Each Xpert MTB/RIF cartridge were Labeled with the sample ID. (Labeling was written on the sides of the cartridge or affix ID label.) The labels on the lid of the cartridge were not written not obstruct the existing 2D barcode on the cartridge.

2. At least 0.5 ml of the total resuspension pellet was transferred to a conical, screw-capped tube for the Xpert MTB/RIF using a sterile transfer pipette. Alternatively, the entire sample might also be processed in the original tube.

3. Re-suspended sediments were storedat 2–8 °C if they were not immediately processed for Xpert MTB/RIF.

4. 1.5 ml of Xpert MTB/RIF Sample Reagent (SR) to 0.5 mL of resuspended sediment sample using a sterile transfer pipette was added and shaked vigorously 10 – 20 times. Note: One back-and-forth movement was a single shake.

5. The specimens' were incubated for 15 minutes at room temperature. At one point between 5 and 10 minutes of the incubation, again shaked the specimen vigorously 10–20 times. Samples were liquefied with no visible clumps of sputum.

Procedure for Preparing the Cartridge

The test must be started within 30 minutes of adding the sample to the cartridge.

1. Using the sterile transfer pipette provided, the liquefied sample was aspirated into the transfer pipette until the meniscus was above the minimum mark.

2. Then the cartridge lid opened. Samplewas transferredinto the open port of the Xpert MTB/RIF cartridge.

3. The cartridge lid was closed for each and inserted in to the machine for analysis. Remaining liquefied sample was kept for up to 12 hours at 2–8 ºC should repeat testing be required. After the cartilages was loaded into the GeneXpert Dx instrument and the test was started within 30 minutes of preparing the cartridge.

Quality Control

Each test includes a Sample processing control (SPC) and probe check (PCC).

Sample Processing Control (SPC): Ensures the sample was correctly processed. The SPC contains non-infectious spores in the form of a dry spore cake that was included in each cartridge to verify adequate processing of MTB. The SPC verifies that lysis of MTB has occurred if the organisms were present and verified that specimen processing was adequate. Additionally, this control detected specimen-associated inhibition of the real-time PCR assay. The SPC was positive in a negative sample and could be negative or positive in a positive sample. The SPC passedmeeting the validated acceptance criteria. The test result will be “Invalid” if the SPC was not detected in a negative test.

Probe Check Control (PCC): before the start of the PCR reaction, the GeneXpert Dx System measures the fluorescence signal from the probes to monitor bead rehydration, reaction-tube filling, probe integrity and dye stability. Probe Check was passed meeting the assigned acceptance criteria.

DECLARATION

I, the undersigned, declare that this proposal is my own work and that all sources of material used for the thesis have been dully acknowledged.

Name: Feleke Mekonnen Demeke

Signature: ----------

Place of submission: Department of Medical Microbiology, School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar.

Date of submission: ----------

This proposal has been submitted for examination with my approval as an advisor.

Advisors:

Dr. Belay Tessema (PhD.)

Signature: _____Date: _____ Place: Gondar, Ethiopia

Dr. Feleke Moges (PhD.)

Signature: _____ Date: _____ Place: Gondar, Ethiopia

Mr. Aschalew Gelaw (BSc, MSc.)

Signature: _____ Date: _____ Place: Gondar, Ethiopia